REVIEW

Inhibition of xperimental cancer cachexia by anti-cytokine and anti-cytokine-receptor therapy

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Abstract

Cachexia consists of a constellation of metabolic changes that occur in cancer patients, including the reduction of muscle and fat tissue, asthenia, anorexia, hypoglycemia and hypercalcemia. These syndromes complicate therapeutic intervention and decrease the quality of life of the patient. This review discusses the involvement of cytokines in cancer cachexia and describes the contribution of IL-6 and other cytokines to the wasting of C-26-bearing mice. The neutralization of IL-6 by antibody, or IL-6 receptor antagonism by suramin, significantly reduce the severity of key parameters of cachexia. The participation of several other factors (PGE2, IL-1, IL-10 and TNF-α) in the cellular communication between the C-26 tumor cell and tumor-infiltrating macrophages is also described.

Key words: *Cachexia; cancer; metabolism; IL-1; IL-6; IL-10; TNF; LIF; macrophage; suramin*

Introduction

Many neoplastic and chronic diseases are associated with multiple metabolic changes collectively known as cachexia. These metabolic changes include a reduction in body fat and muscle tissue, hypoglycemia, anorexia, anemia and asthenia.[1] It has long been recognized that cachexia is a major cause of death in cancer patients[2] and complicates therapeutic intervention.[3] Many cancer patients (up to 50%) exhibit wasting by the time of diagnosis, and nearly all patients have lost a considerable amount of weight at the time of death.[4] Attempts to compensate for the negative caloric balance through total parenteral nutrition have no effect, and occasionally even worsen the progress of wasting.[5] Cachexia is therefore an important aspect of cancer, and its management could improve the quality of life, increase the survival of patients, and allow for more effective anticancer therapy.

To understand the mechanisms involved in wasting, experimental tumor models have been extensively studied. However, only a few rodent tumors are capable of inducing wasting of the host.[6] To mimic clinical cachexia, wasting must occur when the tumor burden is small,[7] which further reduces the number of available tumor models. Many of the studies with rodents involve large tumor burden, where cachexia occurs only at the final stage of the disease. The wasting observed in such cases can be primarily attributed to anorexia and to competition between the host and the tumor for essential nutrients and energy sources.[8] Some commonly used experimental cachexia models in which cachexia appears in the early stage of tumor growth are the XK-1 hypernephroma,[9] the Mac-16 adenocarcinoma,[10] the Yoshida-130 hepatoma[11] and the colon (C)-26 adenocarcinoma[12].

Role of cytokines in cachexia

The involvement of cytokines in cachexia has been the topic of extensive research. This research can be arbitrarily delineated into three distinct experimental approaches. The first involves metabolic changes following the administration of relatively large quantities of a purified recombinant cytokine. In the second category metabolic changes occur after the inoculation of genetically engineered tumor, where a certain cytokine is overexpressed and secreted by the tumor. In the third approach an assessment of the involvement of a constitutively secreted cytokine by a tumor or by the host in response to an unmanipulated tumor is achieved by attempting to neutralize the action of a given cytokine in vivo. It is important to note that in the first two approaches the pathophysiologic role of a given cytokine may be amplified to such an extent as to distort its true function. The introduction of an excess of a single cytokine in vivo may result in a toxic syndrome that should also give rise to weight loss. In principle, the third experimental approach should result in the most dependable information. It is revealing to utilize antibody neutralization experiments in the tumor-bearing host in order to critically evaluate the role of cytokines in the complex events that lead to

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cachexia. It is equally important to establish that the antibody treatment does not reduce tumor burden, but rather improves the condition of the host.

One of the major factors that has been implicated in wasting, termed cachectin, is found in the serum of animals infected with *Trypanosoma.*[13] Cachectin was later found to have an amino acid sequence identical to that of tumor necrosis factor-α (TNF-α).[14] TNF-α/cachectin was shown to effectively inhibit the enzyme lipoprotein lipase (LPL) in 3T3 L1 adipocytes.[15] Inhibition of this enzyme is believed to cause an increase in circulating triglycerides in vivo by decreasing their incorporation into fat and muscle tissue, and appears to correlate with the reduction of fat tissue seen in cancer patients.[16] In addition to hypertriglyceridemia, the direct administration of TNF-α into animals causes hypoglycemia, anorexia and weight reduction.[17] Attempts to mimic chronic wasting by multiple low-dose administrations of TNF-α have failed, because of the development of tachyphylaxis.[18] Interestingly, despite the transient effect of TNF-α on weight loss, the hypertriglyceridemia is still observed even after daily injections of TNF-α. These data suggest that hypertriglyceridemia cannot necessarily be linked to wasting. More recent studies have shown that hypertriglyceridemia is the result of the stimulation of hepatic lipogenesis, rather than the inhibition of LPL.[19] Additional studies have shown that LPL inhibition is not restricted to TNF-α. Other cytokines, including interleukin-1α (IL-1α),[20] interferon-α (IFN-α), IFN-γ,[21] leukemia inhibitory factor (LIF)[23] and IL-6[23] have all been demonstrated to decrease LPL activity. Together, these results suggest that LPL inhibition may not be the cause of wasting seen in cachexia.

Mice bearing tumors transfected to express high levels of TNF-α develop cachexia as compared with animals bearing control tumors.[24,25] In these animals significant weight loss develops only when serum TNF-α levels are high. By contrast, in animals bearing the same tumor where circulating TNF levels are not high, cachexia cannot be detected. In yet another example, when the effect of TNF-α is neutralized with anti-TNF-α antiserum, the cachectic effects are attenuated.[26] However, the treatment also reduces tumor burden, which should also decrease the extent of wasting. Anti-TNF-α antibody treatment of MCG-101-bearing mice has also been shown to attenuate cachexia and to inhibit tumor growth.[27] These data suggest that, depending on the circulating level of TNF-α, the cytokine may cause wasting or, alternatively, may have a beneficial role in controlling tumor growth.

To artificially reproduce the situation of a TNFα-producing tumor, studies involving the continuous infusion of TNF-α have been conducted. These have yielded mixed results. This treatment results in a transient weight loss associated with anorexia in normal rats. The same degree of weight loss, however, can be seen in saline-injected control rats that are pair-fed the same amount of food as the TNF-α-infused rats.[28] This suggests that TNF-α can cause anorexia, but does not induce further weight loss. However, more recent results show that protein content in TNF-α-infused rats is markedly decreased.[29] Hence animals that receive TNF-α have an increased body water and a decreased protein content compared with pair-fed controls.

Rats bearing the Yoshida AH-130 ascitis tumor exhibit a marked decrease in body weight, skeletal muscle and adipose tissue, and these changes correlate with circulating TNF-α.[11] Interestingly, in this model anti-TNF-α antibody attenuates muscle protein degradation but does not prevent weight loss.[30] Additional studies have shown that the intraperitoneal administration of TNF-α causes proteolysis of skeletal muscle,[31] and this proteolysis may be linked to the TNF-α-induction of free ubiquitin in skeletal muscle.[32] The non-lysosomal ubiquitin system for protein degradation may be an important pathway involved in TNF-α-mediated proteolysis. Further investigation will determine the importance of this observation to cancer-related wasting.

An added complexity of the involvement of TNF-α in hypermetabolism is the fact that this cytokine has been recently linked to hypometabolic conditions, for example insulin resistance.[33] In the diabetic Fa/Fa Zucker rat, TNF-α mRNA is upregulated 4–5-fold in adipose tissue, and the TNF-α protein is increased both locally and systemically. In this model anti-TNFα antibody can restore insulin sensitivity, suggesting a linkage between insulin resistance and TNF-α.[34] Insulin resistance has also been documented in gastrointestinal cancer patients with high circulating levels of TNF-α.[35] Insulin resistance in non-insulin-dependent diabetes (NIDDM) can be reversed by several administrations of high doses of insulin.[33] Insulin has indeed been shown to reverse both TNF-α-induced[36] and cancer-induced[37] weight loss, protein catabolism and anorexia. Similarly, the administration of insulin to the Yoshida AH-130 tumor-bearing rats prevents protein loss, as compared with saline-treated animals.[38] At present, it is not known whether the reversal of protein catabolism by insulin involves a simple compensation for insulin resistance or causes a shift in metabolism toward an anabolic pathway. In sum, there is evidence supporting a role for TNF-α in mediating anorexia and protein degradation, but the effect of this cytokine on lipid and carbohydrate metabolism remains quite controversial.

The participation of additional cytokines in cachexia has also been documented. The role of IFN-γ in wasting has been addressed in two experimental models. In MCA-sarcoma- and in Lewis lung-carcinoma-bearing mice, antibody against IFN-γ, but not antibody against TNF-α, has been shown to decrease somewhat the degree of cachexia.[39,40] The potential role of LIF in cachexia has been addressed as well. Nude mice bearing human tumors that produce LIF lose weight,[22] and the implantation of animals with a tumor designed to overproduce LIF results in cachexia as part of a more generalized fatal syndrome.[41] However, it is not reported whether anti-LIF antibody would actually attenuate cancer cachexia. It remains to be established whether other cytokines such as oncostatin M and CNTF are directly involved in cancer cachexia.

Involvement of IL-6 in cancer cachexia and hypercalcemia

An experimental cachexia model has been identified that fulfills the criteria of early onset with a relatively small tumor burden. In at least this tumor model IL-6, but not TNF-α, is involved in the process leading to cachexia.[42] Murine colon (C)-26 adenocarcinoma is an undifferentiated tumor induced by the carcinogen *N*-nitroso-*N*-methylurethane.[43] When injected into syngeneic mice, the C-26 tumor induces an early-onset wasting.[12,42] To obtain a reproducible and manipulable source of cells, a cell line termed C-26IVX was generated. This cell line grows well in culture, retains the transplantability of the original tumor in syngeneic hosts and induces severe weight loss in syngeneic mice.[42] C-26-bearing mice begin to lose weight at tumor masses that are less than 0.7 g. The extent of weight loss in these mice is approximately 35%. A significant reduction in muscle, complete exhaustion of fat stores, hypoglycemia and hypercalcemia are all present in C-26-bearing mice. On the other hand, hypertriglyceridemia and severe anorexia cannot be observed, suggesting a TNF-α-independent mechanism. Several lines of evidence suggests that IL-6 plays a role in the wasting of C-26-bearing mice. First, increasing levels of IL-6 can be correlated with the degress of wasting. Second, resection of the primary tumors decreases serum IL-6 and reverses weight loss. Third, the administration of neutralizing antibody against IL-6, but not against TNF-α, significantly suppresses the development of key parameters of cachexia. Thus anti-IL-6 treatment prevents the wasting of muscle and fat tissues, and significantly reduces the extent of hypoglycemia and hepatic acute phase response.[42] Notably, the antibody treatment does not reduce tumor burden, suggesting that the antibody protects the host directly. It is interesting that neutralizing antibodies against murine LIF, IFN-γ and CNTF do not afford protection against wasting in the C-26-bearing mouse (G Strassmann, unpublished work).

An additional cancer-related paraneoplastic syndrome is hypercalcemia, which represents a serious medical problem.[44] It is widely believed that two major mechanisms contribute to hypercalcemia associated with solid tumors. These include an increase in bone resorption and renal reabsorption by tumor/host derived factors. Several factors, including TNF, IL-1, colony stimulating factor (CSF), transforming growth factor (TGF) and parathyroid hormone-related peptide (PTHrp) have been all implicated in the pathogenesis of hypercalcemia.[45] In parallel with the wasting syndrome, C-26-bearing mice also develop hypercalcemia.[46] In this model neutralizing anti-IL-6 antibody significantly reduces the extent of hypercalcemia.[46] Additional information suggests that IL-6 participates in the pathophysiology that leads to hypercalcemia. IL-6 is implicated in the increase of bone resorption associated with post-menopausal osteoporosis.[47] Both estradiol and anti-IL-6 antibody reduce osteoclast development in culture,[48] and the administration of anti-IL-6 specifically reduces the number of osteoclastic cells in ovariectomized mice.[49] The involvement of IL-6 in cachexia and hypercalcemia is not restricted to the C-26 cachexia model. Nude mice bearing CHO cells transfected with the IL-6 gene also develop cachexia and hypercalcemia.[50] Neutralizing anti-IL-6 antibody significantly reduces wasting and acute phase response in endotoxin-induced[51] and turpentine-induced[52] acute inflammatory reactions. Anti-murine-IL-6 antibodies provide partial but significant protection against a generalized Shwartzman reaction,[53] and anti-human-IL-6 reverses hypercalcemia in nude mice bearing a human carcinoma.[54] Perhaps the most compelling data on the involvement of IL-6 in wasting has recently been described.[55] This research shows that neutralizing anti-human-IL-6 has a clear beneficial effect on the weight loss in AIDS patients with lymphoma.[55] Other studies, however, demonstrate that IL-6 alone does not cause cachexia. Thus no weight loss can be observed in animals injected with IL-6[56] or in mice expressing the IL-6 transgene.[57] Also, in addition to its involvement in metabolic changes, IL-6 has been implicated in several unrelated chronic inflammatory conditions.[58] Thus important questions in the IL-6 field are the nature of the additional factors/cytokines that cooperate or synergize with its action, and how this pleiotropic cytokine contributes to such a wide variety of pathologies. Studies involving the C-26 tumor model may be ideal to define the additional factors that, in concert with IL-6, participate in paraneoplastic syndromes.

Macrophage–tumor cell interaction in the C-26 model

The issue of the cellular source of IL-6 in the C-26 experimental cachexia model has been addressed. Culture fluids of the C-26IVX line produce approximately two orders of magnitude less IL-6 than single-cell suspensions from freshly disaggregated tumor. Macrophages represent an important component of the inflammatory cell infiltrate to a wide variety of human and murine tumors.[59] Because C-26 tumors are infiltrated by macrophages, but not by granulocytes or lymphocytes,[60] we hypothesized that host macrophages may play an important role in augmenting IL-6 production.[60] The testing of this hypothesis may form the basis of the contribution of host immune cells to metabolic changes.

Femtomolar concentrations of IL-1, but not TNF-α, upregulate IL-6 gene expression and production by the C-26 line. The cell line expresses a relatively high number of IL-1 receptor type I. Co-culture of the C-26 line with syngeneic macrophages results in a similar potentiation of tumor IL-6 release. The production of IL-6 in these experiments can be specifically blocked by antibody against IL-1 receptor type I.[60] Further evidence of the

involvement of macrophage-derived IL-1 in potentiating tumor IL-6 release is found in experiments where the intratumoral administration of IL-1 receptor antagonist significantly reduces the extent of cachexia and circulating IL-6 levels.[61] This information suggests that the interaction between the C-26 cells and tumor-infiltrating macrophages contributes to the cachectic effect of the C-26 tumor in vivo. To further study the effects of the C-26-derived IL-6 in vivo, attempts were made to increase IL-6 release by the known IL-6 stimulator, endotoxin. To our surprise, the administration of LPS results in a significant beneficial effect against cachexia.[62] The protection afforded by LPS is abrogated by treating the mice with anti-TNF-α, but not with antibodies against IL-6, IL-1 receptor or LIF. Direct administration of TNF-α into the mice causes a similar protective effect on cachexia. Histological analyses of the tumor shows that both TNF-α and LPS treatments induce hemorrhagic necrosis, which destroyed up to 90% of the tumor mass. Thus the effect of TNF-α against cachexia is generally attributed to its capacity to induce tumor necrosis. As mentioned above, our previous studies show that C-26-infiltrating macrophages are activated to release IL-1. Why then did these macrophages not produce TNF-α? The release of TNF-α would be beneficial to the host by destroying the tumor and preventing the cachexia. This question has a broad implication involving the complex interplay between tumor and host. In an attempt to answer this question, we searched for TNF-α-inhibitory factor in culture fluids from the C-26 tumor. Two inhibitory factors detected in the tumor mass were IL-10 and PGE2. Whereas the C-26IVX line produces PGE2, no detectable amount of IL-10 can be found. Therefore we hypothesized that the IL-10 in the tumor mass is derived from another source. Indeed, when mouse peritoneal macrophages are pretreated with C-26IVX-conditioned medium followed by LPS stimulation, inhibition of TNF-α, but not of IL-1α, release can be seen. Moreover, the inhibitory action of the C-26-derived factor on TNF-α release can be significantly and specifically reversed by neutralizing antibody against IL-10. Thus the C-26IVX line produces a soluble factor that inhibits TNF-α via a mechanism involving macrophage IL-10. Similarly to the C-26-derived factor, the inhibition of TNF-α release by PGE-2-treated and LPS-stimulated macrophages can be significantly reversed by anti-IL-10 antibody.[63] Several additional experiments reveal an identity between the C-26IVX inhibitory factor and PGE2.[62] Collectively, the data suggest that in response to the tumor, tumor-infiltrating macrophages produce IL-1, which upregulates tumor production of IL-6 and PGE2. In turn, tumor-derived PGE2 blocks TNF-α release, in part by elevating macrophage IL-10 levels. These data support the concept that a set of signals is exchanged between the tumor and tumor-infiltrating macrophages. This cellular communication is most likely to the tumor's advantage, involving the release of certain cytokines and the inhibition of others.

Inhibition of C-26 cachexia by suramin

Because the large-scale treatment of cachexia and of other inflammatory conditions with neutralizing anti-cytokine antibody is largely impractical, we searched for small inhibitory compounds of cytokine action. The experimental drug suramin (MW 1429) is a polysulfonated naphthylurea originally developed as an antitrypanosomal and antifilarial agent.[64] More recently, the drug was shown to interfere with the binding of a variety of growth factors, including EGF, FGF, PDGF, TFG-β and IGF-1, to their corresponding cell surface receptors.[65] In addition to its ability to disrupt autocrine/paracrine growth stimulation, suramin modulates the activity of several intracellular enzymes. The drug is also known to inhibit the proliferation of several cancer lines in culture. Primarily for these reasons, suramin is currently being investigated in the clinic for its efficacy as an antitumor agent in treating several inoperable cancers.[65]

We attempted to determine whether suramin is capable of interfering with the binding of IL-6 to its receptor.[66] In culture, suramin inhibits the proliferation of an IL-6-dependent cell line and the binding of radioactive IL-6 to target cells. The interference of IL-6-receptor binding by suramin is further underscored in crosslinking studies. In a dose-dependent manner, suramin interferes with the binding of IL-6 to the gp80 and gp130 subunits of the IL-6 receptor complex.[66] In a dose- and time-dependent manner, the administration of the drug to C-26-bearing mice significantly reduces weight loss, fat depletion and hypoglycemia. The drug does not decrease tumor burden at the same time as it protects against cachexia. Moreover, suramin inhibits the uptake of radioactive IL-6 but not of radioactive TNF-α to the liver. Interestingly, anti-IL-6 treatment did not increase the protection against cachexia provided by suramin.[66] The collective implication of the data is that suramin inhibits cachexia, at least in part by interfering with the binding of IL-6 to its receptor in vivo. The action of suramin in inhibiting cytokine receptor binding and biological activity is not restricted to IL-6. We also found that the drug can inhibit the action of other cytokines. It blocks the stimulation of acute-phase plasma proteins by IL-6-type cytokines (i.e. those cytokines that utilize the gp130 subunit for signal transduction), including LIF, CNTF and IL-11.[67] In addition, suramin interferes with IL-1 receptor binding and blocks IL-1 biological activities,[68,69] and inhibits endotoxin-induced activation of human endothelial cells.[70] The usefulness of suramin in the clinic is yet to be determined, primarily because of its considerable toxicity.[65] Ultimately, then, the identification of suramin as an inhibitor of cytokine receptors may lead to the discovery of other chemicals capable of exerting a more potent and a more selective antagonism of IL-6 in vivo.

Summary

Cachexia consists of a constellation of metabolic changes that occur in cancer patients, including the reduction of

muscle and fat tissue, asthenia, anorexia, hypoglycemia and hypercalcemia. These syndromes complicate therapeutic intervention and decrease the quality of life of the patient.

This review has discussed the involvement of cytokines in cancer cachexia and has described the contribution of IL-6 and other cytokines to the wasting of C-26-bearing mice. The neutralization of IL-6 by antibody, or IL-6 receptor antagonism by suramin, significantly reduce the severity of key parameters of cachexia. The participation of several other factors (PGE2, IL-1, IL-10 and TNF-α) in the cellular communication between the C-26 tumor cell and tumor-infiltrating macrophages has also been described.

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